




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/EP96/02270 <b>(22) International Filing Date:</b> 22 May 1996 (22.05.96) <b>(30) Priority Data:</b> PD95A000101 22 May 1995 (22.05.95) IT <b>(71) Applicant (for all designated States except US):</b> FIDIA ADVANCED BIOPOLYMERS S.R.L. [IT/IT]; Via de' Carpentieri, 3, I-72100 Brindisi (IT). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> BELLINI, Davide [IT/IT]; Via Po, 34, I-35036 Montegrotto Terme (IT). CALLEGARO, Lanfranco [IT/IT]; Via Bravi, 35, I-35020 Ponte di Brenta (IT). <b>(74) Agent:</b> GERVASI, Gemma; Notarbartolo & Gervasi S.r.l., Viale Bianca Maria, 33, I-20122 Milano (IT).		<b>(81) Designated States:</b> AL, AM, AU, AZ, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> A POLYSACCHARIDE HYDROGEL MATERIAL, A PROCESS FOR ITS PREPARATION AND ITS USE IN MEDICINE, SURGERY, COSMETICS AND FOR THE PREPARATION OF HEALTH CARE PRODUCTS		
		
<b>(57) Abstract</b> <p>A polysaccharide hydrogel material consisting of a crosslinked product of a functionalized derivative of alginic acid or hyaluronic acid, whose carboxylic groups are partially esterified with an unsaturated aliphatic or an araliphatic alcohol, and the remaining carboxylic groups are partially salified with an alkaline, alkaline earth metal cation or with tetralkylammonium. This hydrogel material, which is prepared by treatment with radiations selected from UV, <math>\beta</math> and gamma radiations is advantageously used in medicine, surgery, cosmetics and for the preparation of health care products.</p>		

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A POLYSACCHARIDE HYDROGEL MATERIAL, A PROCESS FOR ITS PREPARATION AND ITS USE IN MEDICINE, SURGERY, COSMETICS AND FOR THE PREPARATION OF HEALTH CARE PRODUCTS.

#### FIELD OF THE INVENTION

5 The present invention concerns a polysaccharide hydrogel material consisting of a crosslinked product of a functionalized derivative of alginic acid or hyaluronic acid, a process for its preparation and its use in medicine, surgery, cosmetics and for the preparation of health care products.

#### 10 TECHNOLOGICAL BACKGROUND

Hydrogel materials such as those obtained starting from synthetic polymers such as poly-hydroxy-ethyl-methacrylate (PHEMA) (Holly, F. J. et al., J. Biomed. Mater. Res. 9, 315, 1975), or from semisynthetic derivatives of natural polysaccharides such as the crosslinked  
15 hyaluronic acid derivative with vinyl-sulfone (Balazs, E. A. et al., Blood Coagulation and Fibrinolysis, 1991, 2, 173-178) are used for preventing surgical adherence, in the release of drugs or biologically active proteins, and in tissue repair. It is also known that hydrogels can be obtained from synthetic polymers by ultraviolet radiation  
20 (Amarpreet S. Sawhney et al., Macromolecules, 1993, 26, 581587), or from hyaluronic acid or chondroitin sulfate (Matsuda et al., ASAJO Journal 1992, Slide Forum 3 - Innovations I, 154-157), and these are suitable for use in numerous biomedical applications such as drug release or antiadherence.

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## SUMMARY OF THE INVENTION

Subject of the present invention is a polysaccharide hydrogel material consisting of a crosslinked product of a functionalized derivative of alginic acid or hyaluronic acid, whose carboxylic groups are partially  
5 esterified with an unsaturated aliphatic or an araliphatic alcohol, and the remaining carboxylic groups are partially salified with a cation selected from the group consisting of alkaline, alkaline earth metal cation or with tetralkylammonium.

A further subject of the present invention is the process for  
10 preparing said hydrogel material, which comprises subjecting said functionalized hyaluronic or alginic acid optionally dissolved in an aqueous solution to radiations selected from the group consisting of UV, gamma and  $\beta$  radiations, optionally in the presence of a catalyst. The present invention further relates to the use of said hydrogel  
15 material in medicine, surgery and in the preparation of health care products in the form of fibers, membranes, threads, gauzes, sponges. These hydrogel materials according to the present invention may also be advantageously used as supports for human cell growth, or as coating for blood vessels, artificial organs, and biomedical materials  
20 consisting of polymers such as polyurethanes, polypropylene, polyesters etc.

A further subject of the present invention relates to controlled release medicaments, suitable to be administered by oral, topical, intravenous, intramuscular, or subcutaneous route, containing at least  
25 one active principle principle and, as the vehicle, the polysaccharide hydrogel material according to the present invention. Finally the present invention further relates to cosmetic compositions containing

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the hydrogel materials according to the present invention.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows:

No. 1: the hydrogel material consisting of the crosslinked product of  
5 the hyaluronic acid functionalized derivative with 50 % of carboxylic  
groups esterified with 3-buten-1-ol and 50% of carboxylic group  
salified with sodium, prepared as described in Example 1.

No.2: the hyaluronic acid inner esters (ACP)

No.3: viscous solution of hyaluronic acid outer esters (HYAFF)

10 No.4: solution of hyaluronic acid sodium salt.

#### DETAILED DESCRIPTION OF THE PRESENT INVENTION

The new derivatives according to the present invention present a  
completely different physical structure from that of the previously  
described products, such as hyaluronic acid (European patent No.  
15 0138572), its inner esters (European patent application No. 0341745).  
or outer esters (US patent No. 4,851,521) as reported in Figure 1. In  
particular, the difference between the two hydrogels indicated as No.s  
1 and 2 is evident. Indeed, while the gels constituted by inner esters  
of hyaluronic acid (No. 2) are formed by microparticles of crosslinked  
20 polymers bound together by simple, physical-type bonds, the new  
compounds (No. 1) present a compact, three-dimensional structure  
(wall-to-wall). The latter are, therefore, characterised by greater  
mechanical resistance.

The precursors of the polysaccharide hydrogel materials according to  
25 the present invention, namely the functionalized derivatives of  
hyaluronic or alginic acid whose carboxylic groups are partially  
esterified with an unsaturated aliphatic or an araliphatic alcohol.

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and the remaining carboxylic groups are partially salified with a cation selected from the group consisting of alkaline, earth alkaline metal cation or with tetraalkylammonium a second part salified with alkaline or alkaline earth metals or quaternary ammonium salts are  
5 described in USP 4,851,521 and in EP 251905 respectively. Preferably the hydrogel materials according to the present invention are the crosslinked product of functionalized derivatives of hyaluronic or alginic acid, whose carboxylic groups are partially esterified with aliphatic alcohols such as allyl alcohol, allyl carbinol, 5-hexene-1-  
10 ol, or with araliphatic alcohols such as cinnamyl alcohol and 4-benzyloxy-2-butene-1-ol and the remaining carboxylic groups are salified with sodium.

The preferred hydrogel materials according to the present invention are the crosslinked product of the functionalized derivatives of  
15 hyaluronic acid.

In this case said hyaluronic acid functionalized derivatives are prepared as described in the above mentioned USP 4,851,521 by using as starting material a hyaluronic acid having any molecular weight between 400 and 3,000,000 Daltons, preferably between 150,000 and  
20 1,000,000 Daltons.

Particularly preferred hydrogel materials according to the present invention are the crosslinked products of functionalized derivatives of hyaluronic or alginic acid, wherein 75% of the carboxylic groups are esterified with the above mentioned aliphatic or araliphatic  
25 unsaturarated alcohols and the remaining 25% of carboxylic groups are salified with sodium. Other particularly preferred hydrogel materials according to the present invention are the crosslinked

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products of functionalized derivatives of hyaluronic acid wherein 50% of the carboxylic groups are esterified with the above mentioned aliphatic or araliphatic unsaturated alcohols and the remaining 50% of carboxylic groups are salified with sodium.

- 5 The process according to the present invention may be carried out in the presence or in the absence of an aqueous solution. By the term aqueous solution we mean: purified water, buffers or physiological solutions, such as phosphate buffer or saline solution.

When the reaction is carried out in an aqueous solution of said  
10 hyaluronic or alginic acid derivative, possible concentrations range between 0.01 and 20% (w/w), and more preferably between 0.1 and 15% (w/w).

When the process is carried out in the presence of a catalyst, this is preferably non-toxic radicalic initiator compounds such as 2,2-  
15 dimethoxy-2-phenyl acetophenone or benzoin methyl ether. The exposure time to radiation, in the process according to the present invention should be comprised between 5 minutes and 6 hours, and preferably between 10 and 360 minutes. It has generally been found that by increasing the concentration of the ester derivative of hyaluronic  
20 acid it is possible to obtain the formation of gels after shorter exposure to ultraviolet radiation. The type of aqueous solution used to obtain hydrogels notably affects the viscoelastic properties of these materials, because they belong to the group of polyelectrolytes. As the synthesized gels are derived from a natural polysaccharide  
25 matrix, they belong to the class of compounds with bioplastic and pharmaceutical properties, and as above pointed out they can be used in numerous fields, from cosmetics to surgery and medicine. For

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example, they can be used as films and membranes in various sectors of medicine, such as ophthalmology, dermatology, otorhinolaryngology, neurology, internal and cardiovascular surgery in particular as tissue substitutes and as agents to enable the adhesion of tissue surfaces  
5 (such as severed nerves) or in preventing surgical adherence, when used in the form of fibers or threads they are particularly suitable for surgical suture or, when made into gauzes, they can be used to advantage for wound dressings and finally, when made into sponges, they can be advantageously used for the medications of wounds and  
10 various lesions.

As above pointed out they can also be advantageously used as supports of human cells such as keratinocytes, fibroblasts, osteocytes, chondrocytes, urocytes, stem cells, endothelial cells, Kupfer's and Langerhan's cells. Moreover as above explained they can be  
15 advantageously utilized as coating for organs, such as cardiac valves, or blood vessels, or of biomedical articles such as urologic catheters. As a matter of fact this type of coating improves to a high degree the biocompatibility of the article to be grafted, thereby improving the performance thereof at a biological level.

20 In particular the hydrogel materials are used as coating for blood vessels following coronary angioplasty, in repair following the dissection of blood vessels and the attachment of flaps on the walls of the same, following spontaneous detachment or lesion, and in the sealing of aneurisms.

25 Therefore a further subject of the present invention relates to the process for coating these blood vessels, artificial organs or biomedical articles, which comprises the following steps:



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a) applying a layer of these hyaluronic or alginic functionalized derivatives as such or in the form of an aqueous solution, by means of conventional techniques onto the surface of these blood vessels, artificial organs or biomedical articles,

- 5 b) subjecting the articles to radiations selected from the group consisting of UV, beta or gamma radiation, optionally in the presence of catalysts.

In addition the hydrogel material according to the present invention can be advantageously used as agents to enable cell-cell interactions  
10 and cell-polymer interaction, as they can act as material for cell recruitment, as fillers in replacements for example in breast replacements, as fillers for dental cavities and in cosmetic surgery, as fillers, in place of collagen, for small areas or cavities and in soft tissue.

- 15 Furthermore, as above seen the hydrogel materials according to the present invention can be advantageously used as the vehicle in controlled release medicaments. These medicaments can be in particular administered by oral, topical, intravenous, intramuscular, or subcutaneous route.

- 20 The active principles contained in this medicament are preferably anaesthetic, analgesic, antiinflammatory, vasoconstrictor, antibiotic/antibacterial or antiviral agents, proteins, peptides, growth factor enzymes or mixtures thereof. Further subjects of the present invention therefore relate to the alternative processes for preparing these  
25 medicaments.

The first process encompasses the incorporation of the active principle in the gel by swelling said hydrogel in a dry state in an

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aqueous solution containing this active principle.

In the second process the incorporation of the active principle in the hydrogel material, according to the present invention, comprises mixing the active principle into the solution of said functionalized  
5   hayluronic or alginic acid derivative and then radiating it to obtain a gel. This last method is particularly useful when large molecules are to be incorporated, such as peptides, proteins, growth factors and enzymes, which would find it difficult to penetrate into a gel left to swell in aqueous solution.

10   In particular when this medicament is administered by topical or oral route, it is preferably in the form of a gel.

In particular when the medicaments according to the present invention are administered by the subcutaneous, intramuscular or intravenous route, are preferably in the form of viscoelastic solutions.

15   The medicaments, according to the present invention, when administered by the subcutaneous route, can also be in the form of capsules.

Finally these medicaments, when subcutaneously, intravenously or intramuscularly administered, may also be in the form of microcapsules or microspheres.

20   The cosmetic compositions according to the present invention are preferably in the form of creams.

For purely descriptive purposes, and without being limited to the same, we report hereafter some examples of the preparation of the hydrogels according to the present invention:

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EXAMPLE 1: Preparation of a hydrogel, from a derivative of hyaluronic acid with 50% of its carboxylic functions esterified with 3-butene-1-ol alcohol (allyl carbinol,  $\text{CH}_2=\text{CH}-(\text{CH}_2)_2-\text{OH}$ ) and the remaining 50% salified with sodium

- 5 6.21 gr of tetrabutylammonium salt of hyaluronic acid with a molecular weight of 180,000 Daltons (10 meq) ( USP4,851,521) is solubilized in 248 ml of dimethylsulfoxide (DMSO) at room temperature. To this solution is added 0.675 gr of 4-bromo-1-butene (5 meq) and the solution is left to stand at a temperature of 30°C for 24 hours. A
- 10 2.5% solution (w/w) of NaCl in water is then added and the resulting mixture is poured into 750 ml of acetone, stirring the while. A precipitate is formed which is filtered and washed three times in 100 ml of acetone/water 5:1, three times with 100 ml of acetone and then vacuum-dried for 24 hours at 30°C. 4.17 gr of the desired product are
- 15 thus obtained. Quantitative determination of the allyl carbinol content is performed by gas chromatography after alkaline hydrolysis. The total content of ester groups is conducted in accordance with the saponification method described on pages 169-172 of "Quantitative organic analysis via functional groups", IV Ed., John Wiley and Sons
- 20 Publication. The ester derivative thus obtained is solubilized at room temperature in purified water at a concentration of 100 mg/ml (300 mg of product in 3 ml). The solution is supplemented with 10 microliters of radicalic initiator, while being stirred. The radicalic initiator solution is prepared by dissolving 100 mg of 2,2-dimethoxy-2-phenyl
- 25 acetophenone (ALDRICH) in 0.5 ml of 2-pyrrolidone. The mixture is divided into equal aliquots of 1 ml each and placed in 5-ml china crucibles. The material thus preprepared is exposed to ultraviolet

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radiation (336 nm), using a portable UV lamp, CAMAG model (220v; 0.18A) or a UV lamp Triwood lamp 6/36 sold by Helios Italquartz<sup>R</sup>. Exposure time is 30 minutes.

EXAMPLE 2: Preparation of hydrogel, from a functionalized derivative  
5 of a hyaluronic acid, with 25% of its carboxylic functions esterified with 5-hexene-1-ol alcohol ( $\text{CH}_2=\text{CH}-(\text{CH}_2)_4-\text{OH}$ ) and the remaining 75% salified with sodium

6.21 gr of tetrabutylammonium salt of hyaluronic acid with a molecular weight of 180,000 Daltons (10 meq) (US 4,851,521) , optionally  
10 dissolved in an aqueous solution, is solubilized in 248 ml of dimethylsulfoxide (DMSO) at room temperature. To this solution is added 0.407 gr of 6-bromo-1-hexene (2.5 meq) and the solution is left to stand at a temperature of 30°C for 24 hours. A 2.5% solution (w/w) of NaCl in water is then added and the resulting mixture is poured  
15 into 750 ml of acetone, stirring the while. A precipitate is formed which is filtered and washed three times in 100 ml of acetone/water 5:1, three times with 100 ml of acetone and then vacuum-dried for 24 hours at 30°C. 4.16 gr of the desired product is thus obtained. Quantitative determination of the 5-hexene-1-ol content is performed  
20 by gas chromatography after alkaline hydrolysis. The total content of ester groups is conducted in accordance with the saponification method described on pages 169-172 of "Quantitative organic analysis via functional groups", IV Ed., John Wiley and Sons Publication. The ester derivative thus obtained is solubilized at room temperature in  
25 purified water at a concentration of 150 mg/ml (450 mg of product in 3 ml of water). The solution is supplemented while stirred with 10 microliters of radicalic initiator, prepared as in Example 1. The

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mixture is divided into equal aliquots of 1 ml each and placed in 5ml china crucibles. The material is exposed to ultraviolet radiation as in Example 1, for an exposure time of 20 minutes.

EXAMPLE 3: Preparation of a hydrogel, from a functionalized derivative  
5 of hyaluronic acid with 50% of its carboxylic functions esterified with cinnamyl alcohol ( $C_6H_5CH=CH-CH_2-OH$ ) and the remaining 50% salified with sodium

6.21 gr of tetrabutylammonium salt of hyaluronic acid with a molecular weight of 180,000 Daltons (10 meq) (USP 4,851,521) is solubilized in  
10 248 ml of dimethylsulfoxide (DMSO) at room temperature. To this solution is added 0.985 gr of cinnamyl bromide (5 meq) and the solution is left to stand at a temperature of 30°C for 24 hours. A 2.5% solution (w/w) of NaCl in water is then added and the resulting mixture is poured into 750 ml of acetone, stirring the while. A  
15 precipitate is formed which is filtered and washed three times in 100 ml of acetone/water 5:1, three times with 100 ml of acetone and then vacuum-dried for 24 hours at 30°C. 4.48 gr of the desired product is thus obtained. Quantitative determination of the cinnamyl alcohol content is performed by gas chromatography after alkaline hydrolysis.  
20 The total content of ester groups is conducted in accordance with the saponification method described on pages 169-172 of "Quantitative organic analysis via functional groups", IV Ed., John Wiley and Sons Publication. The ester derivative thus obtained is solubilized at room temperature in purified water at a concentration of 100 mg/ml (300 mg  
25 of product in 3 ml of water). The solution is supplemented with 10 microliters of radicalic initiator. The radicalic initiator solution is prepared by dissolving 100 mg of benzoin methyl ether (ALDRICH) in

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0.5 ml of 2pyrrolidone. The mixture is divided into equal aliquots of 1 ml each and placed in 5-ml china crucibles. The material is exposed to ultraviolet radiation as in Example 1, for an exposure time of 30 minutes.

- 5   **EXAMPLE 4:** Preparation of a hydrogel, from a functionalized derivative of alginic acid with 50% of its carboxylic functions esterified with 3-butene-1-ol alcohol (allyl carbinol,  $\text{CH}_2=\text{CH}-(\text{CH}_2)_2-\text{OH}$ ) and the remaining 50% salified with sodium

4.17 gr of tetrabutylammonium salt of alginic acid (prepared with  
10 alginic acid from *Macrocystis pyrifera*) corresponding to 10 meq of a monomeric unit (Italian patent by Fidia S.p.A., No. 1203814) is solubilized in 248 ml of dimethylsulfoxide (DMSO) at room temperature. To this solution is added 0.675 gr of 4-bromo-1-butene (5 meq) and the solution is left to stand at a temperature of 30°C for 24 hours. A  
15 2.5% solution (w/w) of NaCl in water is then added and the resulting mixture is poured into 750 ml of acetone, stirring the while. A precipitate is formed which is filtered and washed three times in 100 ml of acetone/water 5:1, three times with 100 ml of acetone and then vacuum-dried for 24 hours at 30°C. 2.14 gr of the desired product is  
20 thus obtained. Quantitative determination of the allyl carbinol content is performed by gas chromatography after alkaline hydrolysis. The total content of ester groups is conducted in accordance with the saponification method described on pages 169-172 of "Quantitative organic analysis via functional groups", IV Ed., John Wiley and Sons  
25 Publication. The ester derivative thus obtained is solubilized at room temperature in purified water at a concentration of 100 mg/ml (300 mg of product in 3 ml of water). The solution is supplemented with 10

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microliters of radicalic initiator, prepared as in Example 1. The mixture is divided into equal aliquots of 1 ml each and placed in 5-ml china crucibles. The material is exposed to ultraviolet radiation as in Example 1, for an exposure time of 30 minutes.

5   **EXAMPLE 5:** Preparation of a membrane of a hydrogel from a functionalized derivative, with 50% of its carboxylic functions esterified with cinnamyl alcohol and the remaining 50% salified with sodium

6.21 gr of tetrabutylammonium salt of hyaluronic acid with a molecular  
10   weight of 180,000 Daltons (10 meq) (USP4,851,521), is solubilized in 248 ml of dimethylsulfoxide (DMSO) at room temperature. To this solution is added 0.985 gr of cinnamyl bromide (5 meq) and the solution is left to stand at a temperature of 30°C for 24 hours. A 2.5% solution (w/w) of NaCl in water is then added and the resulting  
15   mixture is poured into 750 ml of acetone, stirring the while. A precipitate is formed which is filtered and washed three times in 100 ml of acetone/water 5:1, three times with 100 ml of acetone and then vacuum-dried for 24 hours at 30°C. 4.48 gr of the desired product is thus obtained. Quantitative determination of the cinnamyl alcohol  
20   content is performed by gas chromatography after alkaline hydrolysis. The total content of ester groups is conducted in accordance with the saponification method described on pages 169-172 of "Quantitative organic analysis via functional groups", IV Ed., John Wiley and Sons Publication. The ester derivative thus obtained is solubilized at room  
25   temperature in purified water at a concentration of 100 mg/ml (1 g in 10 ml of water). The solution is supplemented, while stirring, with 35 microliters of radicalic initiator, prepared as in Example 1. The

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mixture is spread evenly over glass slides measuring  $5\text{cm}^2$ . The material is then exposed to ultraviolet radiation (336 nm), using a portable UV lamp, CAMAG model (2230v; 0.18A), for an exposure time of 2 hours.

- 5 **EXAMPLE 6:** Preparation of a cream containing as active principle the hydrogel of Example 3 based on hyaluronic acid partially esterified with cinnamyl alcohol

A hydrogel prepared according to Example 3 is dried for 48 hours at  $37^\circ\text{C}$  and at  $60^\circ$  for 24 hours, after which it was crushed and sieved  
10 through a 40-100 micron mesh.

100 gr of cream contain:

	dry state hydrogel prepared as described in Ex.3	0.1	g
	Polyethylene monostearate 400	10	g
	Cetiol V	5	g
15	Lanette SX	2	g
	Methyl paraoxybenzoate	0.075	g
	Propyl paraoxybenzoate	0.050	g
	Sodium dihydroacetate	0.100	g
	Glycerine	1.500	g
20	Sorbitol 70	1.500	g
	Test cream	0.050	g
	Sterile water	q.s. to 100	ml

- EXAMPLE 7:** Preparation of an injectable solution containing as its active principle a hydrogel based on hyaluronic acid esterified with  
25 cinnamyl alcohol prepared as described in Example 3



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One 100-ml ampoule contains:

dry state hydrogel prepared as described in Ex.3	0.1	g
Sodium chloride	0.9	g
Sterile water	q.s. to 100	ml

5 **EXAMPLE 8: Preparation of a gelatin capsule containing the hydrogel and carbene oxolone**

The hydrogels described in Examples 1-3 are cut ( $1\text{ cm}^3$ ) and dried for 48 hours at  $37^\circ\text{C}$  and at for 24 hours at  $60^\circ\text{C}$ . The compounds thus dried are left to swell for 24 hours at  $37^\circ\text{C}$  in a solution of carbenoxolone  
10 at a concentration of 50 mg/ml. These processes of drying and drug incorporation were repeated four times on some of the gel samples. This was to increase the quantity of incorporated active principle. The present example also refers to the loading of active principles or drugs other than the one mentioned here.

15 **EXAMPLE 9 Preparation of a hydrogel, with 50% of its carboxylic functions esterified with 3-butene-1-ol alcohol (allyl carbinol,  $\text{CH}_2=\text{CH}-(\text{CH}_2)_2-\text{OH}$ ) and the remaining 50% salified with sodium, by exposure to gamma radiations, in the presence of a radicalic initiator.**

20 The derivative prepared as described in the first part of Example 1, is solubilized at room temperature in purified water at a concentration of 100 mg/ml (300 mg of product in 3 ml water). The solution is supplemented with 10 microliters of a radicalic initiator, while being stirred. This solution is prepared by dissolving 100 mg  
25 2,2 -dimethoxy-2-phenylacetophenone in 0.5 ml of vinyl-2-pyrrolidone. The mixture is placed in a 5 ml glass vial sealed with a stopper and fastened with a metal cap. The material thus prepared undergoes gamma

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radiations at 0.09 Mrad/hr for 4 hours.

EXAMPLE 10 Preparation of a hydrogel from a functionalized derivative of hyaluronic acid, with 25% of carboxylic functions esterified with 3-hexene-1-ol alcohol ( $\text{CH}_2=\text{CH}-(\text{CH}_2)_4-\text{OH}$ ) and the remaining 75%  
5 salified with sodium by exposure to gamma rays in the absence of a radicalic initiator.

The functionalized derivative of hyaluronic acid prepared as described in Example 2, is solubilized at room temperature in purified water at a concentration of 150 mg/ml (450mg of product in 3 ml water). The  
10 mixture is placed in a 5 ml glass vial sealed with a stopper and fastened with a metal cap. The material thus prepared undergoes gamma radiations at 1.25 Mrad/hr for 2 hours.

EXAMPLE 11 - Preparation of a hyaluronic acid hydrogel membrane , from a functionalized hyaluronic acid derivative wherein 50% of  
15 its carboxylic functions are esterified with 3-butene-1-ol alcohol (allyl carbinol,  $\text{CH}_2=\text{CH}-(\text{CH}_2)_2-\text{OH}$ ) and the remaining 50% salified with sodium, by exposure to gamma radiations in the absence of a radicalic initiator.

The functionalized derivative of hyaluronic acid, prepared as  
20 described in Example 1, is solubilized at room temperature in purified water at a concentration of 60 mg/ml (600 mg product in 10 ml water). The solution is spread evenly over a  $5 \text{ cm}^2$  glass slide and left to evaporate spontaneously at  $37^\circ\text{C}$ . The membranes thus obtained undergo gamma radiation at 2.5 Mrad/hr for 2 hours.

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EXAMPLE 12 Preparation of a hydrogel from a functionalized derivative of hyaluronic acid, whose 25% of carboxylic functions are esterified with 3-hexene-1-ol alcohol ( $\text{CH}_2=\text{CH}-(\text{CH}_2)_4-\text{OH}$ ) and the remaining 75% are salified with sodium by exposure to beta rays in the presence of a radicalic initiator.

The functionalized derivative prepared as described in example 2, is solubilized at room temperature in purified water at a concentration of 80 mg/ml (240 mg product in 3 ml water). The solution is supplemented with 10 microliters of the radicalic initiator prepared as described in Example 9. The mixture is placed in a 5 ml glass vial sealed with a stopper and fastened with a metal cap. The material thus prepared undergoes beta radiation at 0.15 KGy.

EXAMPLE 13 : Preparation of a hydrogel membrane, from a functionalized derivative of hyaluronic acid derivative, wherein 50% of carboxylic functions are esterified with 3-butene-1-ol alcohol (allyl carbinol,  $\text{CH}_2=\text{CH}-(\text{CH}_2)_2-\text{OH}$ ) and the remaining 50% salified with sodium by exposure to beta rays in the absence of a radicalic initiator.

The material is prepared according to example 11 and the membranes thus obtained undergo beta radiation at 2.5 KGy.

## CLAIMS

1 1. A polysaccharide hydrogel material consisting of a crosslinked  
2 product of a functionalized derivative of hyaluronic or alginic acid,  
3 whose carboxylic groups are partially esterified with an unsaturated  
4 aliphatic or an araliphatic alcohol, and the remaining carboxylic  
5 groups are partially salified with a cation selected from the group  
6 consisting of alkaline, alkaline earth metal cation or with  
7 tetralkylammonium.

1 2. The polysaccharide hydrogel material according to claim 1, wherein  
2 they are the crosslinked product of functionalized derivatives of  
3 hyaluronic or alginic acid, whose carboxylic groups are partially  
4 esterified with aliphatic alcohols selected from the group consisting  
5 of allyl alcohol, allyl carbinol, 5-hexene-1-ol, or with  
6 araliphatic alcohols such as cinnamyl alcohol and 4-benzyloxy-2-  
7 butene-1-ol and the remaining carboxylic groups are salified with  
8 sodium cation.

1 3. The polysaccharide hydrogel material according to any one of claims  
2 1 and 2 wherein they are the crosslinked product of the  
3 functionalized derivatives of hyaluronic acid, having a molecular  
4 weight ranging from 150,000 and 1,000,000 Daltons.

1 4. The polysaccharide hydrogel material according to any one of claims  
2 1-3, wherein they are the crosslinked products of functionalized  
3 derivatives of hyaluronic or alginic acid having 75% of the carboxylic  
4 groups are esterified with aliphatic or araliphatic unsaturarated  
5 alcohols and the remaining 25% of carboxylic groups are salified with  
6 sodium.

1 5. The polysaccharide hydrogel material according to any one of

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2 claims 1-3, wherein they are the crosslinked products of  
3 functionalized derivatives of hyaluronic acid having 50 % of the  
4 carboxylic groups esterified with the above mentioned aliphatic or  
5 araliphatic unsaturarated alcohols and the remaining 50% of  
6 carboxylic groups salified with sodium.

1 6. A process for preparing the polysaccharide hydrogel material  
2 according to any one of claims 1-4 which comprises subjecting said  
3 functionalized hyaluronic or alginic acid optionally dissolved in an  
4 aqueous solution to radiations selected from the group consisting of  
5 UV, gamma and  $\beta$  radiations, optionally in the presence of a catalyst.

1 7. The process according to claim 6 wherein when it is carried out on  
2 an aqueous solution of said hyaluronic or alginic acid derivative, the  
3 concentration of said functionalized derivative of alginic or  
4 hyaluronic acid ranges between 0.01 and 20% (w/w).

1 8. The process according to claim 7 wherein said concentration of said  
2 functionalized derivative of hyaluronic or alginic in the aqueous  
3 solution range between 0.1 and 15% (w/w).

1 9. The process according to any one of claims 6-8, wherein, when the  
2 process is carried out in the presence of a catalyst this is a non-  
3 toxic radicalic initiator selected from the group consisting of 2,2-  
4 dimethoxy-2-phenyl acetophenone or benzoin methyl ether.

1 10. The process according to any one of claims 7- 10 with exposure  
2 time to radiation comprised between 5 minutes and 6 hours.

1 11 . The process according to claim 10 wherein said exposure times are  
2 comprised between 10 and 360 minutes.

1 12. The polysaccharide hydrogel material according to any one of  
2 claims 1-5, in the form of fibers , membranes, threads, gauzes.

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3 sponges for use in medicine, surgery, and in the preparation of health  
4 care products.

1 13. The polysaccharide hydrogel material according to claim 12, in the  
2 form of films and membranes for use in ophthalmology, dermatology,  
3 otorhinolaryngology, neurology, internal and cardiovascular surgery as  
4 tissue substitutes, as agents to enable the adhesion of tissue  
5 surfaces or in preventing surgical adherence.

1 14. The polysaccharide hydrogel material according to claim 12, in the  
2 form of fibers or threads for surgical suture.

1 15. The polysaccharide hydrogel material according to claim 12 in the  
2 form of gauzes for wound dressings.

1 16. The polysaccharide hydrogel material according to claim 12 in the  
2 form of sponges for use in medications of wounds and various lesions.

1 17. The polysaccharide hydrogel material according to any one of  
2 claims 1-5, for use as the support of human cells selected from the  
3 group consisting of keratinocytes, fibroblasts, osteocytes,  
4 chondrocytes, urocytes, stem cells, endothelial cells, Kupfer's and  
5 Langerhan's cells .

1 18. Coating consisting of the polysaccharide hydrogel material  
2 according to any one of claims 1-5 for blood vessels, artificial  
3 organs, and biomedical materials consisting of polymers selected from  
4 the group consisting of polyurethanes, polypropylene, polyesters.

1 19. The coating according to claim 18, wherein said artificial organs  
2 are cardiac valves and said biomedical articles are urologic  
3 catheters.

1 20. The coating according to claim 18 for blood vessels following  
2 coronary angioplasty, in repair following the dissection of blood

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3 vessels and the attachment of flaps on the walls of the same,  
4 following spontaneous detachment or lesion, and in the sealing of  
5 aneurisms.

1 21. A process for applying the coating according to any one of claims  
2 18-20 onto blood vessels , artificial organs and biomedical articles  
3 comprising the following steps:

4 a) applying a layer of these hyaluronic or alginic functionalized  
5 derivatives as such or in the form of an aqueous solution, by means of  
6 conventional techniques onto the surface of said blood vessels,  
7 artificial organs or biomedical articles.

8 b) subjecting said articles to radiation selected from the group  
9 consisting of UV, beta or gamma radiation, optionally in the presence  
10 of a catalyst.

1 22. The polysaccharide hydrogel material according to any one of  
2 claims 1-5 for use as agents to enable cell-cell interaction and cell-  
3 polymer interaction.

1 23. The polysaccharide hydrogel material according to any one of  
2 claims 1-5, for use as fillers in replacements, and in dental cavities.

1 24. The hydrogel material according to claim 23, wherein said  
2 replacement is breast replacement.

1 25. The hydrogel material according to any one of claims 1-5 for use  
2 in cosmetic surgery as fillers for small areas or cavities and in soft  
3 tissue.

1 26. A controlled release medicament, suitable to be administered by  
2 oral, topical, intravenous, intramuscular, or subcutaneous route  
3 containing at least one active principle and , as the vehicle, the  
4 polysaccharide hydrogel material according to any one of claims 1-5.

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1 27. The controlled release medicament according to claim 26, wherein  
2 said active principle is selected from the group consisting of  
3 anaesthetic, analgesic, antiinflammatory, vasoconstrictor, antibiotic/  
4 antibacterial or antiviral agents, proteins, peptides, growth factor  
5 enzymes and mixtures thereof.

1 28. The controlled release medicament according to any one of claims 26  
2 and 27, wherein, when this medicament is administered by topical or  
3 oral route, it is in the form of a gel.

1 29. The controlled release medicament according to any one of claims 26  
2 and 27 for subcutaneous, intramuscular or intravenous administrations  
3 in the form of a viscoelastic solution.

1 30. The medicament according to any one of claims 26 and 27 for  
2 subcutaneous administration in the form of capsules.

1 31. The medicament according to any one of claims 26 and 27, for  
2 subcutaneous, intravenous or intramuscular administration in the form  
3 of microcapsules or microspheres.

1 32. A process for preparing a controlled release medicament according  
2 to any one of claims 26-31, wherein the incorporation of the active  
3 principle in the hydrogel material is accomplished by swelling this  
4 hydrogel material in a dry state in an aqueous solution containing  
5 this active principle.

1 33. A process for preparing the controlled release medicament  
2 according to any one of claims 26-31, wherein the incorporation of the  
3 active principle in the hydrogel material comprises mixing the active  
4 principle into the solution of said functionalized hyaluronic or  
5 alginic acid derivative and then treating the obtained mixture with  
6 radiation selected from the group consisting of: UV, beta and gamma



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7 radiations, optionally in the presence of a catalyst.

1 34. The process for preparing controlled release medicament according  
2 to claim 33, wherein the active principle is selected from the group  
3 consisting of proteins, peptides, growth factors, and enzymes.

1 35. A cosmetic composition containing the polysaccharide hydrogel  
2 material according to any one of claims 1-5.

1 36. The cosmetic composition according to claim 35 in the form of  
2 a cream.

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FIG. 1

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/02270

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C08B37/04 C08B37/08 C09D105/04 C09D105/08 A61K47/36  
A61K7/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 09176 (CLOVER CONSOLIDATED LIMITED) 13 May 1993  see page 14, line 22 - page 15, line 11 see page 16, line 7 - page 17, line 15 see page 18 - page 19; example 3 see page 29; example 17 see page 30 - page 31; example 19 see page 39; example 29 see claims	1,2,6,9, 12,15, 17,26, 30,31,33
A	WO,A,94 01468 (M.U.R.S.T. ITALIAN MINISTRY FOR UNIVERSITIES AND SCIENTIFIC AND TECHNO) 20 January 1994 see abstract  --- -/--	1,12-16, 25



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

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Date of the actual completion of the international search

4 September 1996

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

International Application No.

PLI/EP 96/02270

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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